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RESEARCH ARTICLE

AMELIORATIVE EFFECTS OF FOUR HERBS (*Withania somnifera, Tinospora cordifolia, Azadirachta indica* AND E CARE SE HERBAL) ON THE PATHOGENESIS OF CHICKEN INFECTIOUS ANAEMIA VIRUS

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ABSTRACT

The present study was conducted to assess the ameliorative effect of four herbal preparations namely *Withania somnifera, Tinospora cordifolia, Azadirachta indica* and E Care Se Herbal on the pathogenesis of chicken infectious anaemia virus (CIAV) in chicks. Ninety day old specific pathogenic free (SPF) chicks were randomly divided into six groups (A to F) in which the first four groups were fed with *W. somnifera* (1% pure extract), *T. cordifolia* (1% pure extract), *A. indica* (0.2% pure extract) and E Care Se Herbal (0.1% in drinking water), respectively, from the first day. Groups E and F were treated as positive and negative controls respectively. Groups A to E were intra-muscularly on 14th day of age with 40 times of 50% chicken infectious dose (CID_{50}) of CIAV A strain. Chicks of each group were sacrificed on 14th day post infection (DPI), the lymphoid organs collected and ameliorative effects of the four herbal preparations were evaluated by assessing the degree of gross and histopathological changes. The chicks of all the herbal treatment groups showed ameliorative effects as revealed by reduced pathological lesions and changes when compared with the positive virus control group. The herbal preparations evaluated in this study can be recommended for immunomodulatory, prophylactic and therapeutic purposes against CIAV induced immunosuppression so as to reduce mortality and secondary infections associated with this virus.

INTRODUCTION

Poultry industry worldwide has been undergoing continuous genetic selection and intensive productions systems that made the birds highly susceptible to various stresses. Such a stressed poultry population is exposed to the opportunistic microorganisms, which evolve into higher pathogenicity levels and the existing pathogens which enhance their virulence. Chicken infectious anaemia (CIA), caused by chicken infectious anaemia virus (CIAV), is an emerging disease of poultry, especially of young chicks, causing considerable economic losses to the poultry industry worldwide (Kataria *et al*., 1999; Todd, 2000; Dhama *et al*., 2002, 2008a, 2011; Verma *et al*., 2005; Schat, 2009). The CIAV causes considerable economic loss to the poultry industry by producing severe immunosuppression, aplastic anaemia and generalized lymphoid atrophy in chicks (McNulty, 1991; Smyth *et al*., 1993; Schat, 2003). This virus is ubiquitous in all major chicken producing countries of the world as evidenced by serology, virus isolation and molecular detection studies (Schat, 2003, 2009; Dhama *et al*., 2003, 2008a; Natesan *et al*., 2006a,b; Toro *et al*., 2006; Kim *et al*., 2010; Bhatt *et al*., 2011a; Snoeck *et al*., 2012; Nayabian and Mardani, 2013). Since CIAV is a potent immunosuppressive agent for very young unprotected chicks, it increases their susceptibility to secondary infections, viz. viral, bacterial and fungal agents, and depresses vaccinal immunity and production performance in the field situations (Pope, 1991; Hu *et al*., 1993; Liu *et al*., 1997; Adair, 2000; Schat, 2003; Miller and Schat, 2004; Basaraddi *et al*., 2013; Gowthaman *et al*., 2013). Like other viral infections there is no specific therapeutic approach for the treatment of CIA infected birds; however, broad spectrum antibiotics are generally used to control or avoid secondary bacterial infections. Birds in convalescent stages can be provided with immunostimulants and hematinics so as to boost the immune system and the process of haematopoiesis, respectively (Schat, 2003; Dhama *et al*., 2008a, 2011). Effective immunomodulatory and therapeutic regimen needs to be evaluated to counter the massive immunosuppression produced by the disease so as to reduce the economic losses produced by this economically important poultry pathogen (Bhatt *et al*., 2011b, 2013; Shyma, 2013). The present study was therefore, undertaken to assess the ameliorative potential of selected herbal preparations viz., *Withania somnifera, Tinospora cordifolia, Azadirachta indica* and E Care Se Herbal on reducing the pathology induced by CIA virus in chicks.

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MATERIALS AND METHODS

Specific Pathogen Free (SPF) chicks

A total number of 100 specific pathogen free (SPF) embryonated eggs, certified free from various poultry pathogens, were kindly

supplied by M/S Venkateshwara Hatcheries Group Pvt. Limited (VHL), Pune, Maharashtra. These eggs were incubated in an isolated setter and hatcher at the Hatchery Unit of Central Avian Research Institute (CARI), Izatnagar. The hatched out White leghorn layer chicks were maintained in experimental infection and control sheds of Avian Diseases Section, Division of Pathology, IVRI, Izatnagar.

Chicken Infectious Anaemia Virus (CIAV)

Virus used in this study was Indian field isolate of CIAV A strain (Pune, Maharashtra) of chicken infectious anemia virus (CIAV) with the accession no: AY583755, which was maintained in Avian Diseases Section, IVRI, Izatnagar.

Herbal preparations

Four herbal preparations were used in this study in order to assess their immunomodulatory and ameliorative potential against CIAV. These were the pure extracts of *Withania somnifera* (Ashwagandha), *Tinospora cordifolia* (Guduchi) and *Azadirachta indica* (Neem) (Natural Remedies Pvt. Ltd., India) and one commercial herbal preparation of poultry named E Care Se Herbal (Provimi India Pvt. Ltd. India).

Experimental design

Ninety (90) clinically healthy day-old SPF chicks were used in the experimental studies. These were divided randomly into six groups A-F, having equal number of chicks (n=15). Groups A to D were fed with respective immunomodulatory herbal preparations as presented in Table 1 while groups E and F were maintained as virus positive and negative controls for viral infection to be given later at 14th day of age. Ashwagandha, Guduchi and Neem were mixed with feed as per the experimental designs of Yamada *et al*. (2011), Rajkumar *et al*. (2009) and Sadekar *et al*. (1998), respectively. E Care Se Herbal was mixed with drinking water as per the product information. All the experimental groups of chicks were reared separately under strict isolated conditions and fed autoclaved feed and water, supplemented with vitamins and minerals, *ad libitum*. The chicks of all the groups were also given antibiotic Lixen (Cephalexin, Glaxo India Ltd.) for five days in prophylactic doses to prevent secondary bacterial infections. They were regularly monitored for clinical signs of disease and mortality, if any. Initially, the chicks of all the groups were kept in Control Experimental Shed, Avian Diseases Section, IVRI, Izatnagar.

At 2 weeks of age, groups A to E were shifted to Experimental Infection shed and were given infection by intramuscular inoculation of 40 times of the 50% chicken infectious doses (CID_{50}) of CIAV A strain. Three chicks from each group were humanely sacrificed on 14th day post infection (DPI) and lymphoid organs (thymus, spleen, liver and bursa of Fabricius) were collected for assessing pathological changes and for confirmation of the establishment of the virus infection by polymerase chain reaction (PCR) testing. Genomic DNA was isolated from the blood and tissues of chicks from all the six groups and DNA was extracted using ® Blood and Tissue Kit (QIAGEN, Germany) following manufacturer's instructions. The primers used for amplification of the VP2 gene of CIAV were F 5'ATG CAC GGG AAC GGC CCA C 3' (forward) and R 5'TCA CAC TAT ACG TAC CCG GG 3' (reverse) generating an amplicon size of 651 bp.

RESULTS

Gross changes

The gross lesions observed in various lymphoid organs of CIAV infected birds at 14th DPI were suggestive of acute form of the disease (Fig. 1). The lesions were severe in group E (virus positive control) with the characteristic generalized lymphoid atrophy. Macroscopically, major findings in chicks of group E revealed atrophied thymus, pale fatty bone marrow, mild to moderately atrophied spleen and bursa, and pale swollen liver. The extent and degree of the gross lesions in the herbal treated groups were lesser compared to the CIAV positive control, suggestive of the protective/ameliorative effects and a resistance to the viral pathogenesis. Organ: body weight ratio taken at 14 DPI in chicks of different experimental groups also indicated ameliorative and immunomodulatory effects of the herbal preparations on CIAV pathogenesis (Table 2).

Histopathology

Histopathological lesions were recorded in various organs viz., liver, thymus, bursa and spleen of CIAV infected chicks, sacrificed at 14th DPI, and are shown in Fig. 2. Lymphoid organs of all the infected groups demonstrated CIAV lesions but the lesions were comparatively milder in herbal treatment groups. In CIAV infected control (Group E), thymus showed atrophy and depletion of lymphocytes in cortex and medulla. In spleen and bursa, there was mild to moderate depletion of lymphocytes. In liver, there was mild degeneration of hepatocytes, dilated blood capillaries and focal lymphoid aggregation. While, Group A treated with *W. somnifera* showed reduction in pathological changes in the tissues examined with mild lymphoid depletions in the thymus and spleen but comparatively no changes in liver and bursa on 14th DPI. Group B treated with *Tinospora* showed much reduction in pathological changes in the tissues with comparatively normal architecture of thymus, mild lymphoid depletion in spleen but comparatively no changes in liver and bursa on 14th DPI. Group C treated with *A. indica* showed mild lymphoid depletion of cortex and severe congestion of medulla in thymus, mild lymphoid depletion in spleen with reduction in white pulp and reticular cell hyperplasia, but comparatively no changes in liver and bursa on 14th DPI. Group D showed comparatively less lesions in thymus and liver and the tissues retained normal architecture. Spleen showed mild lymphoid depletion

Table 1. Experimental Design

Group		No. of birds	Dose (herbal preparation)*	Route
A.	*Withania somnifera* (Ashwagandha)	15	1%	Oral (in feed)
B.	*Tinospora cordifolia* (Guduchi)	15	1%	Oral (in feed)
C.	*Azadirachta indica* (Neem)	15	0.2%	Oral (in feed)
D.	E-Care Se Herbal	15	0.1%	Oral (drinking water)
E.	Positive control	15	-	
F.	Negative control	15	-	

Table 2. Organ : body weight ratio at 14 DPI in chicks of different experimental groups

Organ	Organ : Body Weight Ratio					
	A	B	C	D	E	F
Liver	0.036	0.035	0.034	0.041	0.034	0.038
Thymus	0.00103	0.00109	0.00095	0.00130	0.00082	0.00224
Spleen	0.00087	0.00090	0.00124	0.00115	0.00160	0.00075

Values are presented as mean of organ (g): body weight (g) of three chicks

around peri-arteriolar lymphoid sheath (PALS) with reticular cell proliferation. Bursa showed moderate lymphoid depletion in cortex and medulla.

Fig. 1. Gross lesions in target organs of the experimental groups.

Bone: Group E (virus positive control) depicts the characteristic pale and thin femur bone whereas all the herbal treated groups (A to D) showed lesser degree of pathological changes. Liver: In group E, pale swollen and mottled liver observed but the pathological changes were less severe in all the treatment groups. Spleen: In group E, enlarged spleen depicting acute viral infection whereas in treatment groups almost normal spleen with only mild enlargement seen. Thymus: Group E showed severely atrophied thymus which is the characteristic lesion of CIA, but in all the treatment groups lesser degree of thymic atrophy observed.

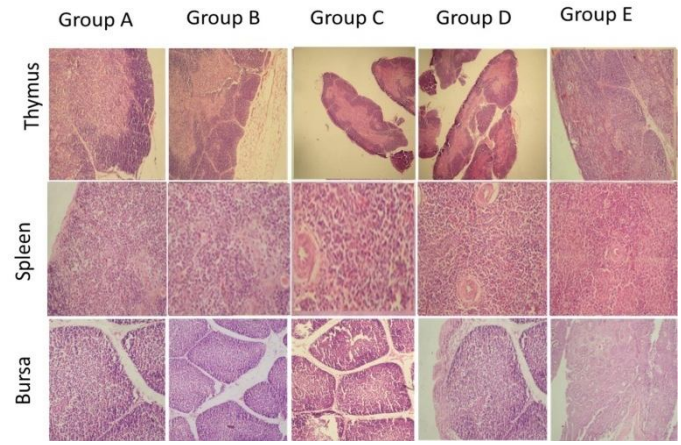

Fig. 2. Microscopic lesions in target organs in experimental groups

Thymus: Chicks of Group E (virus positive control) showed generalized lymphoid atrophy of thymic cortex, while in all the herbal treatment groups there was lesser degree of thymic atrophy. Spleen and Bursa: In group E, there was acute lymphoid depletion whereas in treatment groups only mild lymphoid depletion observed.

Confirmation of virus in target organs by PCR

All the CIAV infected groups (A to E) were found positive for CIAV DNA at 14th DPI. PCR amplified products showed a distinct virus specific DNA band of 651 bp in length in 1% agarose gel electrophoresis (Fig. 3). There was no amplification of DNA from uninfected control chicks (Group F), which was included as negative control.

DISCUSSION

Chicken infectious anaemia (CIA) is an emerging disease of poultry, especially of young chicks, causing considerable economic losses to the poultry industry worldwide (Dhama *et al*., 2002, 2008a; Bhatt *et al*., 2011a). The CIAV causes severe immunosuppression, aplastic anaemia, lymphoid atrophy and predisposes birds to secondary infections (Schat, 2003; Dhama *et al*., 2008a; Dhama *et al*., 2011; Snoeck *et al*., 2012). CIAV possesses specific tropism for lymphocytes, which is responsible for lymphoid depletion in affected birds (Todd, 2000; Schat, 2003, 2009). In addition to immature T cells, mononuclear cells from the spleen are also susceptible to infection. Gross lesions include generalized lymphoid atrophy particularly of thymus, which is the most consistent lesion; and pale and fatty bone marrow, which is the most characteristic lesion (McNulty, 1991; Schat, 2003, 2009; Hoerr, 2010). Liver becomes swollen and mottled with pale/yellowish discoloration. Thymic atrophy sometimes results in an almost complete absence of thymic lobes (Dhama *et al*., 2002, 2008a, 2011; Basaraddi *et al*., 2013).

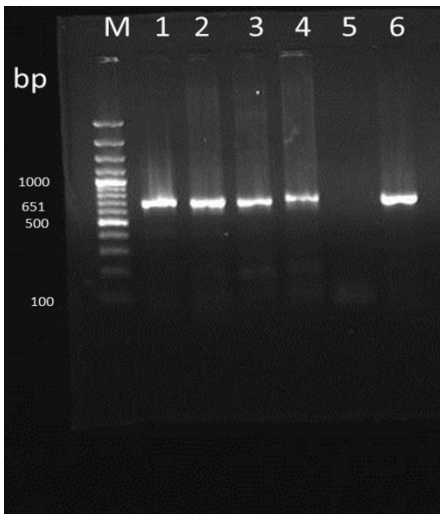

Fig. 3. CIAV PCR testing of thymus from chicks of each group on 14th DPI

Lane M: 100 bp ladder; Lane 1- 4: VP2 amplified products from chicks of herbal treated Groups (A to D, respectively); Lane 5: Negative control (Group F); Lane 6: virus positive control (Group E).

In the present study, group E, representing the virus positive control, depicted the characteristic pathological changes of CIAV infection. Gross and histopathological changes revealed a mild to moderate degeneration and depletion of lymphocytes in the lymphoid organs examined in chicks of group E on 14th DPI, which is in accordance with the findings of Dhama (2002) and Basaraddi (2011). Earlier studies suggest that the primary target cells of CIAV include haematopoietic precursor (haemocytoblasts) and thymic precursor (lymphoblasts) cells in the bone marrow and thymus cortex, respectively (Dhama *et al*. 2002, 2008a; Schat, 2003, 2009; Oluwayelu 2010). Ratio of organ : body weight ratio at 14 DPI also revealed ameliorative potential of the herbal preparations CIAV induced pathogenesis (Table 2). VP2 gene of CIAV was amplified from blood and tissues of all the five infected groups (A-E) confirming the virus multiplication ad establishment of the virus infection in experimental chicks. PCR has been reported to be a very useful molecular tool for diagnosis of CIAV (Dhama *et al*., 2004, 2008a; Kataria *et al*., 2005; Natesan *et al*., 2006a; Oluwayelu, 2010; Basaraddi *et al*., 2013). All the four herbal treated groups have shown better immunumodulatory response against CIAV infection as evidenced by the reduction of gross and histopathological changes in target lymphoid organs. *W. somnifera* has its action on immune system in selectively skewing the immune response towards Th1 response cells rather than Th2 cells by increased IFN gamma, IL-2 versus IL-4 cytokines levels (Bani *et al*., 2006) and it modulates immune response by augmenting the counts of CD4+ cells, CD8+ cells, and natural killer (NK) cells (Davis and Kuttan, 2002; Khan *et al*., 2006). These actions exerted by *W. somnifera* are attributed to effectively resisting the viral multiplication and pathogenic effects in

lymphoid tissues, resulting in significantly reduced viral load and pathogenic lesions in lymphoid organs. Group B treated with *T. cordifolia* revealed less lymphoid lesions which can be correlated with the property of this plant in augmenting the myeloid differentiation of bone marrow progenitor cells and the recruitment of macrophages (Rege *et al*., 1999; Dhanukar *et al.*, 2000; Singh *et al*., 2006), thereby it can enhance the cell mediated immune response in chicks. This can be accounted for the lesser degree of pathological changes in group B compared to that of group E.

Reduced histopathological lesions and viral load of CIAV in tissues of *A. indica* fed chicks (Group C) points to the potent antiviral properties and immunopotentiating effect of this plant which coincides with the findings of Haq *et al*. (1999) and Ansari *et al*. (2012) that *A. indica* was found to be effective on humoral and cell mediated immune responses, in broilers which had survived an outbreak of infectious bursal disease and Newcastle disease. The findings are also in accordance with Renu *et al*. (2003) who concluded the enhanced cell mediated immune response of Neem leaf extract by delayed type of hypersensitivity (DTH) in terms of increased skin thickness to 2, 4-dinitro-chlorobenzene in skin contact sensitivity test and enhanced humoral immune response against NDV antigen by indirect ELISA. Along with herbal remedies, early and rapid diagnosis as well as effective and novel vaccines and therapeutics for combating chicken infectious anaemia virus and other poultry pathogens is required. This strategy would help in combating CIA, an economically important immunodepressive disease of poultry and other associated secondary infections, which would help alleviate economic losses to the poultry farmers and producers/industry (Dhama *et al*., 2004; Kataria *et al*., 2005; Dhama *et al*., 2008a,b; Mahima *et al*., 2012; Dhama *et al*., 2013a,b,c,d; Tiwari *et al*., 2013).

Conclusion

The present study revealed that the herbal preparations viz., *W. somnifera, T. cordifolia, A. indica* and E Care Se Herbal were effective in the amelioration of CIAV induced pathological lesions in chicks. Combination of these herbal preparations may also be evaluated in future for use against CIAV.

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REFERENCES

Adair, B.M., 2000. Immunopathogenesis of chicken anemia virus infection. *Dev. Comp. Immunol*., 24: 247-255.

Ansari, J., Khana, S.H., Haqb, A. and Yousaf, M. 2012. Effect of the levels of *Azadirachta indica* dried leaf meal as phytogenic feed additive on the growth performance and haemato biochemical parameters in broiler chicks. *J. Applied Anim Res.*, 9: 1-10.

Bani, S., Gautam, M., Sheikh, F.A., Khana, B., Satti, N.K., Suri, L.A., Qazi, G.N. and Patwardhan, B. 2006. Selective Th1 up-regulating activity of *Withania somnifera* aqueous extract in an experimental system using flow cytometry. *J. Ethnopharmacol*., 107: 107–115.

Basaraddi, M. 2011. Evaluation of cell mediated immune response to chicken infectious anemia virus (CIAV) in experimentally infected chicks. M. V. Sc. Thesis, Deemed University. Indian veterinary Research Institute (IVRI), Izatnagar, (U.P.), India

Basaraddi, M.S., Dhama, K., Wani, M.Y., Sawant, P.M., Tiwari, R., Kumar, D., Singh, S.D. and Singh, R. 2013. Downregulation in cytokines profiles and immunopathological changes in chicks infected with chicken infectious anaemia virus. *African J. Microbiol. Res.*, 7: 2464-2474.

Bhatt, P., S.K. Shukla, K. Dhama and A.K. Thathoo, 2011b. Amelioration of chicken infectious anaemia virus induced immunosuppression by protein and immunoglobulin supplementation in chicks. *Vet. Practitioner*, 12: 90-93.

Bhatt, P., S.K. Shukla, M.Y. Wani, R. Tiwari and K. Dhama, 2013. Amelioration of chicken infectious anaemia virus induced immunosuppression by immunomodulator and haematinic supplementation in chicks. *Vet. Arhiv*, (In Press)

Bhatt, P., Shukla, S.K., Mahendran, M., Dhama, K., Chawak, M.M. and Kataria, J.M. 2011a. Prevalence of chicken infectious anaemia virus (CIAV) in commercial poultry flocks of northern india: a serological survey. *Transbound. Emerg. Dis*.,58:458–460.

Davis, L. and Kuttan, G. 2002. Effect of *Withania somnifera* on cell mediated immune response in mice. *J. Exp. Clin. Cancer Res*., 21: 585-590.

Dhama, K, K. Karthik, S. Chakraborty, R. Tiwari, S. Kapoor, A. Kumar and P. Thomas, 2013a. Loop-mediated isothermal amplification of DNA (LAMP) – a new diagnostic tool lights the world of diagnosis of animal and human pathogens: a review. *Pak. J. Biol. Sci*. doi: 10.3923/pjbs.2013.

Dhama, K. 2002. Pathogenecity and immunosuppressive effects of chicken infectious anaemia virus (CIAV) in chicks and evaluation of diagnostic tests for its detection. Ph. D. Thesis, Deemed University. Indian veterinary Research Institute (IVRI), Izatnagar, (U.P.).

Dhama, K., J.M. Kataria, N. Senthilkumar and S. Tomar, 2004. Differentiation of Indian isolates of chicken anaemia virus (CAV) by polymerase chain reaction (PCR)–restriction enzyme (RE) analysis. *Indian J. Comp. Microbiol. Immunol. Infect. Dis*., 25: 75-79.

Dhama, K., J.M. Kataria, N.S. Kumar, S. Tomar, B.B. Dash and G.R. Ghalsasi, 2003. Immunosuppressive effects of the Indian isolate of chicken anemia virus (CIAV) in specific pathogen free chicks. *Indian J. Poult. Sci*., 38: 185-194.

Dhama, K., Kataria, J.M., Dash, B.B., Senthilkumar, N. and Tomar, S. 2002. Chicken infectious anemia (CIA): A review. *Ind. J. Comp. Microbiol. Immunol. Infect. Dis*., 23: 1-15.

Dhama, K., M. Mahendran, P.K. Gupta and A. Rai, 2008b. DNA Vaccines and their applications in Veterinary Practice: Current Perspectives. *Vet. Res. Commun.* 32: 341-56.

Dhama, K., Mahendran, M., Somavanshi, R. and Chawak, M. M. 2008a. Chicken infectious anaemia virus: An immunosuppressive pathogen of poultry – A review. *Ind. J. Vet. Path.*, 32: 158-167.

Dhama, K., S. Chakraborty and R. Tiwari, 2013c. Panchgavya therapy (Cowpathy) in safeguarding health of animals and humans – A review. *Res. Opin. Anim. Vet. Sci*., 3: 195-208.

Dhama, K., S. Chakraborty, M.Y. Wani, R. Tiwari and R. Barathidasan, 2013d. Cytokine therapy for combating animal and human diseases – a review. *Res. Opin. Anim. Vet. Sci*., 3: 195-208.

Dhama, K., S. Chakraborty, Mahima, M.Y. Wani, A.K. Verma, R. Deb, R. Tiwari, and S. Kapoor, 2013b. Novel and emerging therapies safeguarding health of humans and their companion animals: a review. *Pak. J. Biol. Sci*., 16: 101-111.

Dhama, K., Singh, S.D., Somvanshi, R. and Kataria, J.M. 2011. Chicken infectious anaemia: (an economically important immunodepressive viral disease of poultry). ICAR-NAE Project Publication, IVRI, Izatnagar, pp: 1-36.

Dhanukar, S.A., Kulkarani, R.A. and Rege, N.N. 2000. Pharmacology of medicinal plants and natural products. *Indian J. Pharmacol.* 32: 81.

Gowthaman, V., S.D. Singh, K. Dhama, R. Barathidasan, M. Asok Kumar, P.A. Desingu, N.K. Mahajan and M.A. Ramakrishnan (2013). Fowl Adenovirus (FAdV) in India: evidence for emerging role as primary respiratory pathogen in chickens. *Pak. J. Biol. Sci*., 15: 900-903.

Haq, A., Meraj, K. A. and Rasool, S. 1999. Effect of supplementing A*llium sativum* (garlic) and A*zadirachta indica* (neem) leaves in

broiler feeds on their blood cholesterol, triglycerides and antibody titre. *Int. J. Agricult. and Biol.*, 1: 125-127.

Hoerr, F.J. 2010. Clinical aspects of immunosuppression in poultry. *Avian Dis*., 54: 2-15.

Hu, L.B., Lucio, B. and Schat, K.A., 1993. Depletion of $CD4^+$ and $CD8^+$ T lymphocyte subpopulation by CIA-1, a chicken infectious anemia virus. *Avian Dis.*, 37: 492-500.

Kataria, J.M., Mohan, M.C., Dey, S, Dash, B.B. and Dhama, K. 2005. Diagnosis and immunoprophylaxis of economically important poultry diseases: a review. *Indian J. Anim. Sci*., 75: 555-567.

Kataria, J.M., Suresh, R.P., Verma, K.C, Toroghi, R., Kumar, N.S., Kataria, R.S. and Sah, R.L., 1999. Chicken infectious anemia (CIA) in India: detection of the agent by polymerase chain reaction and transmission study. *Indian J. Comp. Microbiol. Immunol. Infect. Dis*., 20: 91-95.

Khan, B., Ahmad, S.F. and Bani, S. 2006. Augmentation and proliferation of T lymphocytes and Th-1 cytokines by *Withania somnifera* in stressed mice. *Int. Immunopharmacol.,* 6: 1394-1403.

Kim, H.R., Kwon, Y.K., Bae, Y.C., Oem, J.K. and Lee, O.S., 2010. Molecular characterization of chicken infectious anemia viruses detected from breeder and broiler chickens in South Korea. *Poult. Sci.*, 89: 2426-2431.

Latheef, S.K. 2013. Evaluation of immunomodulatory properties of four herbal preparations (*Withania somnifera, Tinospora cordifolia, Azadirachta indica* and E Care Se Herbal) against Chicken Infectious Anaemia in chicks. M.V.Sc. Thesis, Deemed University. Indian veterinary Research Institute (IVRI), Izatnagar, (U.P.). India.

Liu, Z.G., Zheng, S.M., Xu, Y.B., Zhou, Z.Y., Wang, X.R., Yang L.P., 1997. Immunopathological changes of chicken infectious anaemia. *Scientia Agricultura Sinica*, 30: 74-82.

Mahima, A. Rahal, R. Deb, S.K. Latheef, H.A. Samad, R. Tiwari, A.K. Verma, A. Kumar and K. Dhama, 2012. Immunomodulatory and therapeutic potentials of herbal, traditional / indigenous and ethnoveterinary medicines. *Pak. J. Biol. Sci.*, 15: 754-774.

McNulty, M.S. 1991. Chicken anaemia agent: a review. *Avian Pathol.*, 20: 187-203.

Miller, M.M. and Schat, K.A., 2004. Chicken infectious anaemia virus: an example of the ultimate host-parasite relationship. *Avian Dis.*, 48: 734-745.

Natesan, S., Kataria, J.M., Dhama, K., Bhardwaj, N. and Sylvester, A., 2006b. Anti-neoplastic effect of chicken anemia virus VP3 protein (apoptin) in Rous sarcoma virus-induced tumours in chicken. *J. Gen. Virol*., 87: 2933-2940.

Natesan, S., Kataria, J.M., Dhama, K., Rahul, S., Baradhwaj, N., 2006a. Biological and molecular characterization of chicken anaemia virus isolates of Indian origin. *Virus Res*., 118: 78-86.

Nayabian, H. and Mardani, K., 2013. Molecular characterisation of the chicken anemia viruses isolated from broiler farms of west Azerbaijan, Iran. *Avian Pathol.*, DOI:10.1080/03079457.2013. 766668.

Oluwayelu, D.O. 2010. Diagnosis and epidemiology of chicken infectious anemia in Africa. *African J. of Biotech.*, 9: 2043-2049.

Pope, C.R., 1991. Chicken anemia agent. *Vet. Immunol. Immunopathol*., 30: 51-65.

Rajkumar. R.S., Yadav, A.S., Kirupasanker, M., Saxena, V.K. and Singh, S. 2009. Effect of *Tinospora cordifolia* supplementation on immunity of broiler chicks. *Indian Vet. J*., 86: 1244-1245.

Rege, N.N., Thatte, U.M. and Dahanukar, S.A. 1999. Adaptogenic properties of six rasayana herbs used in Ayurvedic medicine. *Phytotherapy Res*., 13: 275-291.

Renu, S., Rakha, N.K., Sandeep, G. and Mishra, S.K. 2003. Effect of Neem (*Azadirachta indica*) leaf extract administration on immune responses of broiler chickens. *J. Immunol. and Immunopathol.* 5: 47-50.

Sadekar, R.D., Kolte, A.Y., Barmase, B.S. and Desai, V.F. 1998) Immunopotentiating effects of *Azadirachta indica* (Neem) dry leaves powder in broilers, naturally infected with IBD virus. *Indian J. Exp. Biol.*, 36:1151-1153.

Schat, K.A. 2003. Chicken infectious anaemia. In: Diseases of Poultry, 11th edn. Y.M. Saif *et al*., (Editors), Iowa State University Press, Ames, IA, Ch. 7, pp: 182-202.

Schat, K.A. 2009. Chicken infectious anaemia. *Curr. Topics in Micro. and Immunol.*, 331: 151-183.

Singh, S. M., Singh, N. and Shrivastava, P. 2006. Effect of alcoholic extract of Ayurvedic herb *Tinospora cordifolia* on the proliferation and myeloid differentiation of bone marrow precursor cells in a tumor-bearing host. *Fitoterapia*, 77: 1-11.

Smyth, J.A., Moffett, D.A., McNulty, M.S., Todd, D., Mackie, D.P., 1993. A sequential histopathologic and immunocytochemical study of chicken anaemia virus infection at one day of age. *Avian Dis.*, 37: 324-338.

Snoeck, C.J., Komoyo, G.F., Mbee, B.P., Nakoune, E. and Faou, A.L. 2012. Epidemiology of chicken anemia virus in Central African Republic and Cameroon. *Virol. J.*, 9: 189.

Tiwari, R., S. Chakraborty, K. Dhama, M.Y. Wani, A. Kumar and S. Kapoor, 2013. Wonder world of phages: potential biocontrol agents safeguarding biosphere and health of animals and humans - current scenario and perspectives. *Pak. J. Biol. Sci*. doi: 10.3923/pjbs.2013.

Todd, D. 2000. Circoviruses: immunosuppressive threats to avian species: a review. *Avian Pathol*., 29: 373-394.

Toro, H., Ewald, S. and Hoerr, F.J., 2006. Serological evidence of chicken infectious anemia virus in the United States at least since 1959. *Avian Dis*., 50: 124-126.

Verma, S., R.C. Katoch, A. Mahajan, M. Sharma, V. Katoch, J.M. Kataria and K. Dhama, 2005. Confirmation of an outbreak of chicken infectious anaemia in organised poultry farm by polymerase chain reaction. *Indian Vet. J.*, 82: 119-122.

Yamada, K., Hung, P., Park, T.K., Park, P.J. and Lim, B.O. 2011. A comparison of the immunostimulatory effects of the medicinal herbs Echinacea, Ashwagandha and Brahmi. *Journal of Ethnopharmacology*, 137: 231-235.

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